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An analysis of primate gait on multiple and inconsistent substrates in natural environments, using *Saimiri sciureus* as a model

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An analysis of primate gait on multiple and inconsistent substrates in natural environments, using *Saimiri sciureus* as a model

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Abstract

An analysis of primate gait on multiple and inconsistent substrates in natural environments, using *Saimiri sciureus* as a model

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Primate quadrupedal gait has primarily been studied in the laboratory setting on artificial poles of different diameters (simulating arboreal locomotion) and on flat surfaces (simulating terrestrial locomotion). However, wild primates encounter complex matrices of substrates of different sizes, orientations, heights, and compliances, and often move on multiple substrates within each locomotor bout. Thus, the current understanding of primate gait is limited by the artificial setting of the laboratory. This study investigates the quadrupedal gait of wild *Saimiri sciureus* (common squirrel monkey) across multiple substrates at the Tiputini Biodiversity Station in Yasuní Biosphere Reserve, Ecuador. High-speed video footage (120fps, 1080p) was collected between August and October 2017. Multiple substrate use was categorized into seven different challenge types including gap, linear transition, obstacle, offshoot, parallel, ladder, and no challenge. The Anteroposterior Sequence method (Abourachid, 2003) was used to calculate time lags between touchdowns of paired fore and hindlimbs and between ipsilateral fore and

hindlimbs (Abourachid, 2003). *Saimiri* used significantly more asymmetrical walking gaits than symmetrical gaits (p=0.02), and, when using symmetrical gaits, used significantly more diagonal sequence than lateral sequence gaits (p<0.01). Alterations of kinematic variables between strides without challenges to strides with challenges were calculated to determine how *Saimiri* kinematically adjusts to challenges during quadrupedal bouts. *Saimiri* significantly adjusted pair lag (p=0.03) and fore lag (p=0.02) in strides with a challenge. *Saimiri* was significantly more likely to change its footfall sequence during strides with a challenge than strides without a challenge (p=0.05). However, type of challenge did not significantly affect kinematic adjustments. These results indicate that primate quadrupedalism is flexible and can be adjusted for animals to maintain stability across complex substrates. This study highlights the importance of combining field and laboratory methods to capture the range of substrate variation that primates face in their natural habitats.

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Introduction

Eadweard Muybridge first identified primates' unique quadrupedal gait when he photographed a walking baboon and noticed the difference in its footfall pattern compared to nonprimate mammals (Muybridge, 1887). Milton Hildebrand (1967) later developed methods for measuring distinct gaits in animals and systematized the primate pattern of symmetrical walking gait as diagonal sequence (DS). DS gaits are characterized by a movement pattern in which a forelimb touches down on the substrate after the diagonally opposite, or contralateral, hindlimb (e.g., left-hind, right-fore; right-hind, left-fore). DS gaits contrast with lateral sequence (LS) gaits that are most commonly used by other quadrupeds, wherein a hindlimb is followed by the touchdown of its ipsilateral forelimb (e.g., left-hind, left-fore, right-hind, right-fore) (Hildebrand, 1980). Diagonal couplets (DC) are commonly used by quadrupeds, meaning the fore-hind footfalls on opposite sides of the body are coupled together in timing (Hildebrand, 1980). Animals that use diagonal sequence, diagonal couplet (DSDC) gaits, such as primates, face biomechanical challenges compared to other gait types, including increased risk of interlimb interference during walking (Hildebrand, 1967, 1980; Larson, 1998; Shapiro and Raichlen, 2005) and reduced balance of the animals' center of mass due to a less optimized support polygon (Vilensky and Larson, 1989; Cartmill et al., 2002). As such, primate locomotor biomechanical studies remain dedicated to understanding the use, function, and evolution of primate DSDC gait (Hildebrand, 1967; Cartmill et al., 2002, 2007; Lemelin et al., 2003; Shapiro and Raichlen, 2005; Lemelin and Schmitt, 2007;

Stevens, 2006, 2008; Wallace and Demes, 2008; Nyakatura et al., 2008; Schmidt, 2008; Lemelin and Cartmill, 2010; Nyakatura and Heymann, 2010; Shapiro et al., 2011; Young, 2012; Young and Shapiro, 2018), but the reason for the evolution of the distinctive primate gait pattern remains not well understood.

Primate gait may have evolved as a way to exploit terminal branches. It has been suggested that primates, acting as seed dispersers, coevolved with angiosperms during the Eocene, and thus evolved adaptations to feed in the terminal branches of fruiting trees, also known as the "fine branch niche" (Sussman, 1991; Sussman et al., 2013). The angiosperm hypothesis and the conceptualization of the fine branch niche has contributed to the way that primatologists think about positional behavior (Grand, 1972; Dunbar and Badam, 2000; Orkin and Pontzer, 2011), and gait specifically (Cartmill et al., 2002; Schmitt and Lemelin, 2002; Stevens, 2006; Lemelin and Schmitt, 2007; Nyakatura and Heymann, 2010; Shapiro and Young, 2010; Toussaint et al., 2015). DSDC gait may be safer for primates during arboreal quadrupedalism in terminal branches because the leading forelimb is able to test a novel substrate while the hindfoot, which grasps more securely and for longer during stance phase than the primate forefoot, is grasping in a protracted position on an already established stable support (Cartmill et al., 2002, 2007; Patel et al., 2015). However, rodent gait research has documented the same benefit for LS gaits used by rats – LS allows a "soft-contact phase" during which the forelimb can explore the stability of an upcoming substrate while the hindlimb is reliably grasping a previously tested substrate (Clarke, 1995; Bolton et al., 2006). Additionally, non-primate arboreal mammals have been shown to adequately navigate relatively narrow substrates

in their arboreal habitats using LS gaits (White, 1990; Lammers, 2007; Shapiro and Young, 2010, 2012; Shapiro et al., 2014; Karantanis et al., 2018). An alternate hypothesis for the evolution of primate DSDC gait suggested that primates may possess a higher degree of forelimb cortical control for exploring new substrates compared to nonprimates due to differences in cerebral pathways that control limb muscular activity and locomotor movements, resulting in a preference for rostro-caudal ipsilateral paired limb movements, or DSDC gaits (Vilensky, 1989; Vilensky and Larson, 1989). However, evidence that some mammals without primate cerebral pathways use DSDC (e.g., marsupials) (White, 1990; Pridmore, 1994; Lemelin et al., 2003; Lemelin and Schmitt, 2007) contradicts a strictly neurological based hypothesis. More research on arboreal stability, specifically within the fine-branch niche, is warranted to further elucidate the debated advantages and disadvantages of DSDC gait, and to provide insight into which gait pattern is most beneficial for navigating multiple substrates.

Despite disagreement over the specific evolutionary pressures selecting for DSDC gait in primates, the influence of substrate characteristics on preferred gait and other kinematic variables remains of keen interest (see Table 1 for definitions of kinematic variables used in this study). Laboratory research has dominated primate gait kinematics studies, resulting in a limited understanding of how complex and natural substrates influence gait. Small-bodied mammals have been the focus of many gait studies, as they are thought to most accurately represent the primate ancestral condition (Lemelin and Schmitt, 2007; Nyakatura and Heymann, 2010; Shapiro and Young, 2010; Shapiro et al., 2014, 2016; Hesse et al., 2015; Karantanis et al., 2015; Young et al., 2016). Laboratory

quadrupedal gait and kinematic studies typically have primates walk on flat platforms to simulate terrestrial locomotion and poles of various diameters and orientations to simulate arboreal supports (Schmitt, 2003; Hanna et al., 2006; Nyakatura et al., 2008; Wallace and Demes, 2008; Young, 2009, 2012; Carlson and Demes, 2010; Shapiro et al., 2011, 2016). Laboratory research on primates indicates that DSDC gaits are not used explicitly in response to decreases in substrate diameter relative to body size, nor explicitly to substrate orientation (Stevens, 2008; Shapiro et al., 2011). Rather, DSDC gait has been shown to be flexible enough to be used across various substrate sizes and orientations, and has been shown to be preferred on a range of substrate sizes and orientations (Schmidt, 2005; Stevens, 2008; Carlson and Demes, 2010; Shapiro et al., 2011). However, substrate orientation has been shown to affect limb phase, duty factor, and degree of limb protraction and retraction (Prost and Sussman, 1969; Nyakatura et al., 2008; Stevens et al., 2011; Hesse et al., 2015), while the effects of substrate diameter on kinematics are more subtle (Schmitt, 2003; Stevens, 2008). Gait type (symmetrical vs. asymmetrical) appears to be more variable on flat surfaces than on poles (Wallace and Demes, 2008; Shapiro et al., 2011). Thus, primate gait type and kinematics likely respond to the interaction between diameter, orientation, and substrate type, rather than to each variable independently.

Field locomotion studies document that free-ranging primates often encounter a complex matrix of substrates including both single and multiple substrates and substrates of various diameters, inclinations, materials (e.g., hardwood trees, lianas, palm fronds, trunks), compliances, and heights (see Table 2 for field studies that include multiple

substrate use) (Hunt et al., 1996; Dunbar and Badam, 2000; Bezanson, 2017). Multiple substrate use is a fundamental concept for understanding the evolution of primate locomotion and should be considered as an adaptive pressure under which primate gait evolved. Although underrepresented in the literature, examining gait on complex substrates that resemble supports primates use in their natural habitats is critical for determining gait flexibility (Nyakatura and Heymann, 2010). Researchers have done this in two ways: 1) introduced laboratory animals to complex substrates, beyond poles and platforms (e.g., multiple substrates, moving substrates, or ladders) (Stevens, 2006; Higurashi et al., 2009), or 2) implemented laboratory methodologies (i.e., videography) in the field setting (Isler and Thorpe, 2003; Nyakatura and Heymann, 2010; Blanchard and Crompton, 2011; Cheyne, 2011; Guillot, 2011; Shapiro et al., 2011; Stevens et al., 2011; Youlatos and Gasc, 2011).

This study aims to understand the effects of multiple/inconsistent substrate use on the gait sequence of *Saimiri sciureus* to gain insight into the adaptive pressures of the complex arboreal habitat on primate gait. Primate gait flexibility is tested by examining the effect of a substrate inconsistency, or a challenge, on wild primate gait kinematics. Both laboratory and field methods are merged by using high-tech laboratory videography methods in the natural field setting. Changes, or adjustments, of quadrupedal kinematics are quantified when primates use multiple substrates and encounter challenges in their environment during a locomotor bout. Challenges analyzed herein include: obstacles, linear transitioning from one substrate to another, using different substrates for multiple footfalls including offshoots, laterally oriented (ladder), or parallel substrates, and

crossing gaps (Table 3). *Saimiri sciureus* is used as the model taxon because they are dedicated arboreal quadrupeds that utilize the lower forest strata, thus making them an opportune species for filming in the wild.

Research questions, hypotheses, and predictions

Q1. Do primates adjust quadrupedal gait kinematics when faced with substrate challenges?

H1. Quadrupedal gait kinematics will differ between challenged and unchallenged strides.

P1-a: Primates will adjust gait kinematics in strides with challenges by changing the timing of limb touchdowns (TD) and liftoffs (LO).

P1-b: Symmetrical gaits will be used most often and will be flexible enough to be maintained during quadrupedalism on complex multiple substrates, as shown in Stevens (2006). Footfall sequence will shift depending on the timing of step events due to challenges. Exaggerated kinematic adjustments will lead to asymmetrical gaits, but asymmetrical gaits will be used less frequently than symmetrical gaits.

P1-c: Forelimb kinematic variables will be adjusted more so than hindlimb variables because forelimbs will be used as exploratory limbs with a "soft-contact phase" to test uncertain substrates.

Q2. Does the type of challenge influence the kinematic adjustments made by primates to maintain quadrupedal walking?

H2. Challenge type will influence the degree to which quadrupedal kinematics are affected.

P2. Kinematic adjustments will range from delaying TD or LO, to changing footfall sequence depending on the type of challenge encountered in the stride. More pronounced challenges, such as gaps and obstacles, will require more exaggerated

kinematic adjustments than subtle challenges, such as linear transitions.

Materials and Methods

STUDY SITE

The Tiputini Biodiversity Station (TBS) is located within the Yasuní Biosphere Reserve, Orellana Province, Ecuador (0°37'S, 76°10'W) (Marsh, 2004; Blake et al., 2012). TBS includes *ca.* 650 hectares of primary lowland *várzea* and *terra firma* tropical rainforest along the northern bank of the Tiputini River with ~30km of trails that allow researchers access to primate habitat (Marsh, 2004; Blake et al., 2012). Mean annual precipitation at Yasuní Research Station (~30km WSW of TBS) is about 3,100mm (Blake et al., 2012).

STUDY SPECIES

Saimiri sciureus (common squirrel money) inhabits a vast geographic range throughout the Amazon basin from Ecuador and Northern Colombia, to Eastern and Central Brazil (Thorington, 1985).The conservation status of *S. sciureus* is listed by the IUCN Red List as "Least concern," but populations are declining (IUCN, 2000). As primate habitats are degraded by anthropogenic forces it becomes increasingly important to understand the relationship between animals and their habitats (Estrada et al., 2017).

Morphology and behavior

Adult *S. sciureus* range in size from 500g-1kg (Smith and Jungers, 1997), and primarily feed on insects and fruit (Lima et al., 2003), though will exploit other available resources such as small vertebrates. *Saimiri* typically occupies lower to middle forest strata (Fleagle and Mittermeier, 1980; Fleagle et al., 1981; Mittermeier and van

Roosmalen, 1981; Marsh, 2004; Pinheiro et al., 2013), and frequently utilize lianas during travel (Fleagle and Mittermeier, 1980). On average, 50% of *Saimiri*'s activity budget is spent foraging and 30% is spent travelling (Pinheiro et al., 2013). Quadrupedal locomotion (walking or running) is the most frequent locomotor mode during travel (55% of bouts), feeding (87% of bouts), and foraging (75% of bouts) (Fleagle and Mittermeier, 1980).

Study group at TBS

S. sciureus at TBS is classified as *S. s. macrodon* and is one of ten known primate species that inhabit TBS (Marsh, 2004). *Saimiri* were often observed in large groups estimated to be between 30 and 50 individuals, which is consistent with what has been documented for groups living in continuous forests (Pinheiro et al., 2013), and for the groups at TBS (Montague et al., 2014). *Saimiri* at TBS were sometimes observed in association with *Cebus albifrons* (white-fronted capuchin), *Callicebus discolor* (red titi monkey), and *Saguinus tripartitus* (golden-mantled tamarin). Data on adults and juveniles were collected; no infants were observed. Due to their prior habituation, use of lower forest strata, and preference for quadrupedal walking, *S. sciureus* at TBS were the perfect study taxon for this research.

DATA COLLECTION

As part of a larger project on nine species at TBS (Dunham et al., *in press*) data on *Saimiri* analyzed for this study were recorded on fifteen nonconsecutive days between August 5 and October 19, 2017. Prior habituation and large group size made it possible

for large amounts of data to be collected in relatively few days. Videos were recorded using a modified GoPro Hero 5 camera equipped with a varifocal zoom lens for high-resolution footage (1080 p resolution, 120 frames-per-second) at distances up to ~50 meters (see Dunham et al., *in press* for detailed methods overview). Lightweight, collapsible tripods were used to stabilize the cameras during filming. *Saimiri* were filmed opportunistically, thus the sample analyzed does not represent a comprehensive overview of the species' quadrupedal locomotion. All data collection procedures complied with Ecuadorian laws and the Institutional Animal Care and Use Committees of the University of Texas at Austin and Northeast Ohio Medical University.

DATA ANALYSIS

This study applies Abourachid's (2003) Antero-Posterior Sequence (APS) method of gait analysis, which allows analysis of both symmetrical and asymmetrical gaits using the same method (Table 4). Abourachid (2003) presents two strong arguments for the utility of the APS method: 1) the bilaterality of all vertebrates results in morphological and functional similarities between the limbs of a pair (fore and hind), and 2) locomotion is activated in a rostro-caudal pattern, which results in the forelimbs being activated for locomotion prior to the hindlimbs. The APS method is advantageous for analyzing animals' responses to irregularities in the environment because the forelimbs approach and react to the challenge prior to and independent of the hindlimbs, and has proven useful for analyzing gait transitions when animals are presented with challenges (Abourachid, 2003; Abourachid et al., 2007; Maes et al., 2008). The APS method's use of

rostro-caudal analysis rather than caudo-rostral as in Hildebrand's model is particularly important for analyzing primate gait due to primates' distinctive aspects of forelimb morphology and function. Distinct primate forelimb features are components of a "compliant" quadrupedal gait including increased joint flexibility, higher degrees of forelimb protraction compared to other quadrupeds, and a reduction in forelimb peak vertical forces compared to hindlimbs (Schmitt, 1999; Larson et al., 2000; Hanna et al., 2006). Therefore, the APS method is advantageous specifically for analyzing primate gait by identifying forelimb movements and determining the benefits of their biomechanical characteristics for locomotion.

The APS method classifies gait based on the succession of step events in time and space. Therefore, an anteroposterior sequence (APS) is defined by Abourachid (2003) as the association of TD and LO cycles of the forelimbs followed by the cycles of the hindlimbs. An APS can be visualized as a stride cycle beginning with the TD of the first forelimb and ending with the TD of the last hindlimb. A locomotor bout is an occurrence of locomotion from the beginning of its movement to finish. In this study, a bout refers to the quadrupedal sequence of locomotion captured by video, which may or may not represent the entire locomotor bout performed by the primate due to difficulty following the animals with the camera for extended periods of time. Kinematic gait variables calculated are defined in Table 1. Six kinematic variables were calculated using the APS method: the time lag between the paired hindlimb touchdowns (hind lag), the time lag between the paired forelimb touchdowns (fore lag), the time lag between ipsilateral limbs on either side of the body (pair lag), forelimb duty factor, hindlimb duty factor, and cycle

duration (Hildebrand, 1977; Abourachid, 2003). Relative speed was calculated as body lengths per second by digitizing the following points in a single frame in BoutKeeper: the animal's length from nose tip to base of tail, and stride length from a reference limb's initial TD to its subsequent TD in the same APS. Relative body length was then calculated by dividing body length in pixels by stride length in pixels. Relative speed was calculated by dividing relative body length by stride duration in seconds, resulting in values with units of body lengths per second. Time lags are calculated as a percentage of the cycle duration of the entire stride, using the first forelimb TD as the reference limb (Abourachid, 2003). Kinematic adjustments were quantified by calculating the change of each kinematic variable from a stride with no challenge to a stride with a challenge. If a temporal lead change occurred between strides (i.e., the order of paired footfalls shifted), the time lag of the limb pair in which the change occurred was negative. Temporal lead changes were used to identify changes in footfall order during a bout.

Videos were analyzed if they contained more than one APS in the quadrupedal bout, and if the animal encountered a challenge or used multiple substrates within the bout. See above and Table 3 for definitions of challenges. Challenges were assigned to each limb in each stride within a bout because not every limb encountered the same challenge in each stride. Therefore, each stride could have multiple challenges, and bouts often contained multiple challenges. If a stride contained multiple challenges, or multiple limbs encountered a challenge, only a single challenge type could be analyzed and was assigned to the challenge type encountered by a forelimb. Quadrupedal walking bout kinematics were analyzed in the open-source MATLAB software, BoutKeeper, which is

a modified version of GaitKeeper (Dunham et al., *in press*) designed for analyzing bouts of quadrupedal locomotion using the Abourachid method (Figure 1). Substrate characteristics for each locomotor bout were qualitatively scored and entered into BoutKeeper during analysis. Qualitative substrate characteristics included substrate diameter (small, medium, large), orientation (vertical, horizontal, decline), and type (liana, branch, frond, trunk, ground) (Figure 2). Substrate diameters were scored qualitatively relative to the individual animal's hand size or trunk diameter, as follows: small: less than the circumference of the animal's hand; medium: greater than the animal's hand, but less than the diameter of the animal's trunk; large- greater than the diameter of the animal's trunk. Orientation was scored qualitatively based on angle of the substrate relative to the ground, but due to difficulties scoring this variable from video footage, it was not used in analysis.

Categorical gait types were assigned to each stride (Table 5). A perfectly symmetrical stride would have a value of 50% of the cycle duration for both fore and hind lags. For this study, strides were considered symmetrical walks if both the hind lag and the fore lag were between 40% and 60%, and the mean duty factor of all of the limbs was 50% or above. A stride was considered a diagonal sequence walk if the mean pair lag was <50% and a lateral sequence walk if mean pair lag was >50%. Asymmetrical walks (referred to here as "rambles") were defined as gait sequences for which fore lag or hind lag were below 40% or above 60% (i.e., asymmetrical), but duty factor was still greater than 50% (walk).

STATISTICAL ANALYSES

Statistical analyses were done in R (R Core Team, 2017). A chi squared test was used to test for differences in frequencies of gait type (symmetrical vs. asymmetrical), and gait sequence (DS vs. LS). Due to small sample sizes, a Fisher's Exact test was used to test if the frequency of temporal lead changes differed for strides with and without a challenge, and then across challenge types per stride, followed by post-hoc pairwise analyses. Post-hoc multiple pairwise comparisons were corrected using the false discovery rate method (Benjamini and Hochberg, 1995). R packages used include: RVAideMemoire (Hervé, 2018) and rcompanion (Mangiafico, 2018).

The change in time lag variables between strides (within a bout) before and after a challenge was calculated, and then a one-way ANCOVA was used to test for the effect of substrate challenges on (change in) time lag variables, with relative speed as the covariate. Both time lags and relative speed were log transformed for analysis. A change in pair lag after encountering a challenge indicated that the animal's coordination between its fore and hind limbs was interrupted, and therefore the timing between those touchdowns was affected. A change of fore lag or hind lag after encountering a challenge indicates that a challenge affected the timing of touchdowns of paired fore or hind limbs, respectively. ANCOVA (with relative speed as the covariate) was also used to test whether challenge *type* (e.g., gap, obstacle, etc.) influenced gait kinematics before and after the challenge was encountered by the animal. Due to small sample sizes for some categories of challenge type, the effect of challenge type was retested with ANCOVA after regrouping into three more evenly sampled challenge type categories: gap, other

(combined data for obstacles, linear transitions, parallel, offshoot, and ladder), and no challenge. R packages used include: lme4 (Bates et al., 2015), dplyr (Wickham et al., 2017), lmerTest (Kuznetsova et al., 2017), and emmeans (Lenth, 2017).

Results

Saimiri used asymmetrical gaits significantly more often than symmetrical gaits across strides and bouts, X^2 (1, N=115) = 5.4348, p =0.019 (Table 5). 58.34% of total strides were asymmetrical, while 37.5% were symmetrical. Additionally, DS symmetrical gaits were used significantly more than LS, X^2 (1, N=45) = 16.2, p <0.05 (Table 5). Strides with challenges were associated with greater frequencies of temporal lead changes than strides without challenges (Fisher's exact test, p=0.05) (Figure 3). Challenge type had a significant effect on frequency of temporal lead changes when all challenge types were considered (Fisher's exact test, p=0.05) (Figure 4), but there were no significant pairwise associations across challenge types. Challenge types were then combined to make sample sizes comparable across categories and were grouped as gap, other, and no challenge. Frequencies of temporal lead changes were significantly different across grouped challenge types (p<0.01) (Figure 5). A post-hoc multiple pairwise comparisons indicated that gap was significantly more likely to cause a temporal lead change than no challenge (p=0.027) and more likely to cause a temporal lead change than "other" challenge types, but this comparison did not reach statistical significance (p=0.08).

The shift in ipsilateral footfall timing (log pair lag) between strides was significantly greater between strides without challenges followed by strides with challenges, than in two consecutive strides without challenges F(1,43)=1.0378, p=0.03 (Figure 6). Additionally, the difference in touchdown timing between the two contralateral paired forelimbs (log fore lag) was significantly greater when a challenge

was confronted in the subsequent stride, compared to when consecutive strides had no challenges; therefore there was a significant effect of challenge presence on log fore lag after controlling for log relative speed, $F(1,53)=5.4792$, $p=0.02$ (Figure 7). All other kinematic variables also increased in the presence of a challenge but the effect of challenges was not statistically significant (Table 6). Type of challenge had no significant effect on log pair lag or log fore lag (Figures 8, 9). This may be due to low sample sizes across different challenge categories (Table 3). When challenge types were grouped as gap, no challenge, and other to evenly distribute sample size, the influence of type of challenge on log pair lag and log fore lag change approaches significance ($p=0.075$ and $p=0.06$, respectively) (Figures 10, 11).

Discussion

Results from this study indicate that the quadrupedal gait of *S. sciureus* is flexible enough to be maintained during multiple substrate use and in strides with substrate challenges, but gait kinematics are influenced by challenges during multiple substrate use. Surprisingly, and contrary to predictions, the primates in this analysis used significantly more asymmetrical walking gaits than symmetrical walking gaits, which is not consistent with what has been documented in laboratory studies. This suggests that symmetrical gait may not be flexible enough to be maintained across changing or complex substrates as shown in Stevens (2006). Notably, when primates did use symmetrical gait, DS was used significantly more frequently than LS. Rambles (i.e., asymmetrical walks) may be more flexible for substrate and gait transitions than DS or LS, which is perhaps why results here contradict laboratory studies that find primates prefer DS symmetrical walking during single substrate use. It is possible that laboratory studies historically may not have captured either naturalistic gait variation or the prevalence of asymmetrical gait.

Fore lag and pair lag were significantly adjusted in response to challenges. As predicted, temporal forelimb adjustments were more prominent than those of the hindlimbs. The significant adjustment of fore lag in response to challenges may reflect primates' morphological and biomechanical forelimb differences compared to other quadrupeds (Schmitt, 1999; Larson et al., 2000). Increased joint flexibility and protraction, as well as decreased vertical forces on the forelimbs may contribute to primates' ability to adjust forelimb kinematics while walking in complex environments

with the hindlimbs safely grasping a reliable support. Significant fore lag adjustment in response to challenges may support the hypothesis that forelimbs are used to explore the stability of a new substrate while the hindlimbs support the animal's body and kinematics remain fairly consistent and stable. Pair lag change in response to various challenge types indicates that primates adjust timing of ipsilateral touchdowns, and thus quadrupedal gait, to react to inconsistencies in their environments. A more comprehensive sample of challenge types is necessary to address the effect of challenge type on primate gait kinematics. Laboratory research has not adequately investigated primate locomotor transitions across complex substrates, and therefore kinematic data for comparisons is not available (but see Stevens, 2006 and Higurashi et al., 2009).

Substrate challenges in certain bouts were found to bring about shifts in temporal limb leads as predicted. In this sample, a temporal lead change never occurred in a stride with no challenge. Therefore, the presence of a challenge significantly influenced a change in footfall order within a stride. Contrary to what was predicted, substrate challenge type effect on temporal lead change failed to reach significance during post-hoc pairwise comparisons. After grouping challenge types, gaps proved to be more likely to result in a substrate challenge, but this could be due to skew in sample size. It remains unclear whether challenge type or another factor in strides with challenges results in temporal lead changes and further investigation is warranted.

The APS model has been used to analyze locomotor transitions of canines in response to obstacles (jumps along a flat runway surface) by Abourachid and colleagues (2007). This study demonstrated that dogs change from symmetrical to asymmetrical

gaits after encountering obstacles by decreasing both fore lag and hind lag, indicating a switch to in-phase coordination within each limb pair (Abourachid et al., 2007). Interestingly, Abourachid et al.'s (2007) results differ from the findings of this study, where fore lag significantly *increased* upon encountering a challenge, indicating a decrease of in-phase coordination within forelimbs. Due to primates' increased forelimb protraction compared to other quadrupeds, increasing fore lag may be an opportune strategy for primates to further increase their support polygon when navigating complex substrates. Additionally, primates' grasping hands and feet may make it possible for primates to maintain stability while decreasing the coordination of paired limbs. Thus, primates' kinematic adjustments in this study may act to increase balance and stability in their arboreal habitat.

Previous gait studies in primates have primarily used Hildebrand's (1980) model for analysis, with the exception of Druelle and colleagues (2017). Druelle et al. (2017) used the APS model to analyze the effect of ontogeny on quadrupedal kinematics of captive *Papio anubis* to elucidate the relationship between interlimb coordination and morphological and dynamical properties of primate limbs and bodies. Similarly, this study used interlimb coordination to understand kinematic shifts as responses to environmental factors. The APS method was particularly pertinent for this study as it allowed different gait types to be analyzed using the same method and thus captured gait transitions and adjustments of kinematic variables. Additionally, the APS method is ideal for analyzing locomotor bouts with challenges because changes of footfall order are captured by differences of the time lag variables, rather than resulting in errors due to

negative values.

Laboratory researchers that have attempted to investigate the relationship between gait kinematics and complex aspects of substrates have found interesting results that elucidate the biomechanical adjustments primates may use to respond to various environmental inconsistencies. Complex substrates (i.e., unstable/moving substrates) may influence gait choice and transitions more so than substrate diameter or inclination (Stevens, 2006; Young et al., 2016). For example, Stevens (2006) described that the type of quadrupedal gait used by primates is influenced by timing of touchdown (TD) and liftoff (LO), which in turn is influenced by substrate stability. For example, a prolonged stance duration of one limb brought about by a moving substrate or an obstacle along a continuous substrate, could result in a switch of footfall sequences due to its effects on the movement of the other limbs. On moving substrates, primates have been shown to maintain symmetrical gait, but shift limb phase, which at times results in a switch from DSDC to LSDC gaits (Stevens, 2006). Additionally, substrate compliance significantly affects gait kinematics, specifically of the hindlimbs (higher hindlimb duty factor, longer hindlimb contact duration, and increased relative hindlimb lead interval), but research on the topic is sparse (Young et al., 2016). Multiple substrate use has been replicated using a horizontal ladder in the laboratory with *Macaca fuscata*, which preferred DSDC gaits to increase stability during quadrupedalism (Higurashi et al., 2009). This study also found that *M. fuscata* transitioned to a relatively unique gait, a two-one sequence (nearly simultaneous TD of the two forelimbs and only one TD of each hindlimb per stride), to increase stability (Higurashi et al., 2009). Further investigation of primate gait across

complex substrates in the laboratory is necessary to quantify kinematic adjustments in response to environmental factors.

Traditional field positional behavior studies have been critical in documenting the interactions of behavior, locomotion, and environment in natural habitats in order to improve our understanding of realistic adaptive pressures for primate gait and locomotion. Field researchers use the broad category "quadrupedal walking" which Hunt and colleagues defined as "locomotion on top of supports angled at <45°; typically all the four limbs contact the support in a particular sequence" (Hunt et al., 1996: 375). Typically, field researchers do not provide details on the gait sequence type (asymmetrical vs. symmetrical, diagonal sequence vs. lateral sequence) due to challenges with documenting such details without videography. Field studies on substrate use across taxa and life history stages suggest that a complex relationship exists between locomotor behavior and substrate (McGraw, 1996; Thorpe and Crompton, 2005; Bezanson, 2009; Dunham, 2015) calling critical attention to the influence of ecology on locomotion. Despite multiple substrate use being part of primate locomotion, it is not frequently reported nor analyzed in positional behavior studies resulting in a lack of understanding of the influence of complex matrices of substrates on primate behavior. Some researchers create informative categories to signify when a primate is utilizing multiple substrates (i.e., terminal branches, fork, clump, multiple) so that readers know multiple substrates are being used, but the details about substrate type, size, orientation, etc. may neither be included nor completely accurate, and are often not consistent across studies (Bezanson et al., 2012). Table 2 identifies 22 field positional behavior studies published between

1996 and 2016 that record and report multiple substrate use. According to Bezanson (2017), 63 total field positional behavior studies were published in that time (see Bezanson, 2017 for complete list of positional behavior studies since 1996). It is critical that field researchers document details about the substrates that primates use as well as how different substrates are used to increase our understanding of the relationship between an animal and its environment (Ripley, 1967; Bezanson, 2017). Additionally, analyzing all substrates used during positional behavior, rather than solely documenting the main weight bearing support, will elucidate positional behavior of the study species and their interactions with available substrates (Thorpe and Crompton, 2005).

Recently, both field and laboratory locomotion/gait researchers have called for increased efforts in integrating research methods and theoretical frameworks to expand the concept of primate gait plasticity and the relationship between gait and ecology (Nyakatura and Heymann, 2010; Vereecke et al., 2011; Bezanson, 2017). Bringing laboratory methods to the field is now realistic given technological advances (Dunham et al., *in press*). Compact, consumer-grade cameras are now comparable to laboratory-grade camera resolution and frames per second and can be transported and used in primate natural habitats to film gait. Application of videography methods in the field are already enlightening our understanding of wild primate gait and substrate use, despite being a relatively recent achievement. For example, field-based gait analysis of *Pongo pygmaeus albeii* revealed that most individuals preferred DSDC gait, independent of age and sex category (Isler and Thorpe, 2003), thus corroborating lab studies that have shown primate preference for DSDC gait (Hildebrand, 1967). In a comparative study of laboratory and

field kinematic gait data for *Saimiri boliviensis*, Shapiro and colleagues (2011) found that primates exhibit greater limb phase flexibility on natural substrates and concluded that laboratory substrates fail to capture the actual flexibility of primate gait, thus encouraging that natural substrates be emphasized in gait studies. In addition to the influence of substrate variability on gait, further integrating lab and field techniques will allow researchers to investigate the relationship between gait and other ecological factors, such as social behavior, foraging, or vigilance.

Conclusion

This study demonstrated that primates are well adapted to navigating complex substrate transitions within their arboreal environments. Primate quadrupedal walking is a flexible locomotor type that primates can adjust depending on the transitional demands of the animal's environment. This study demonstrated that DSDC walking may not be best suited for primates moving across multiple substrates, but rather, asymmetrical walks may better accommodate complex substrates than do symmetrical walks. Asymmetrical walking may be an energetically conservative way to maintain a steady, slow pace while also maintaining balance and stability on inconsistent substrates. Laboratory gait studies do not capture the variation of primate quadrupedalism by failing to document asymmetrical walking gaits, either because of experimental design or filtering of data to focus on symmetrical gaits. Primate gait studies would benefit from not filtering experimental samples to only include symmetrical gaits, as studying asymmetrical kinematics may enlighten our understanding of complex substrate use and locomotion in the wild. Future research is needed to further investigate the complicated relationship between primate quadrupedal kinematics and environment. Specifically, recreating multiple substrate use in the laboratory would allow the expansion of this analysis with additional precise methods, such as force plates, 3D footage, and measurements of joint angles. Future field kinematic studies will also further our understanding of locomotion within primates' natural and adaptive context. This study was limited by the opportunistic footage that was captured, and thus is not representative of the array of challenges and types of substrate use that *S. sciureus* encounters regularly. Moving forward, field

research that combines observational data collection of the comprehensive positional behavior repertoires combined with videographic kinematic data will provide a more comprehensive grasp of locomotor plasticity and will expand our understanding of kinematics of various locomotor modes.

Appendices

APPENDIX 1: TABLES

Table 1: Definitions of kinematic variables.

Variable	Definition	Unit	Citation
Cycle duration	Total time it takes for all four limbs to touch down in a stride sequence, relative to first limb TD in a stride	s	Abourachid, 2003
Pair Lag	Time lag between the footfall of forelimb and ipsilateral hindlimb expressed as % of total cycle duration	%	Abourachid, 2003
Fore Lag	Time lag between forelimb footfalls expressed as % of total cycle duration	%	Abourachid, 2003
Hind Lag	Time lag between hindlimb footfalls expressed as % of total cycle duration	%	Abourachid, 2003
Duty factor (DF)	% of stride duration during which a forelimb or hindlimb contacted the substrate (i.e., stance phase duration scaled to total stride duration)	%	Hildebrand, 1977
Stride frequency	The reciprocal of stride duration	Hz	Hildebrand, 1977
Relative stride length	Distance the animal traverses along the substrate during a stride relative to its body length	m	Dunham et al., *in press*
Relative speed	Product of relative stride length and stride frequency	$\%s^{-1}$	Dunham et al., *in press*
Symmetry	% of stride duration separating subsequent TDs within a limb girdle; used to distinguish symmetrical and asymmetrical gaits	%	Hildebrand, 1977
Temporal lead change	Change in order of TD of either pairs of limbs, identified by a negative hind lag, fore lag, or pair lag value	%	This study

Table 2: Field studies that report multiple substrate use since 1996.

Adapted from Bezanson, 2017.

Publication	**Species**	**Details on multiple substrate use collected (methods)**	**Data presented (results)**
Walker and Ayres, 1996	*Cacajao calvas calvas*	identify single support, double supports, and several supports	proportion of activity budget that took place on each number of supports
Warren and Crompton, 1997	*Avahi occidentalis, Lepilemur edwardsii*	identify initial and terminal support diameters and orientations (for leaps) - most notably, fork (two or more large supports)" and "foliage (multiple fine supports)"	percentage of substrate type used per locomotor mode
Bergeson, 1998	*Alouatta palliata, Cebus capucinus, Ateles geoffroyi*	records number of supports used	percentages of multiple branch use during feeding
Dagosto and Yamashita, 1998	*Propithecus diadema, Eulemur rubriventer, Eulemur fulvus*	t = smallest substrate size category, primates described to usually use several of these	percentage of use per species
Walker, 1998, 2005	*Pithecia pithceia, Chiropotes stanas*	records "mix" substrate use = "multiple supports of various diameters	percentages of "mix" for different types of sitting and leaping
Garber and Rehg, 1999	*Cebus capucinus*	terminal branches = "mass of small branches orientation at various angles to the ground"	percentage of use of "terminal" by hands/feet and tail
Cant et al., 2001	*Ateles belzebuth, Lagothrix lagothrica*	identify if animals use single or multiple supports	proportion of locomotion that used multiple supports
Garber and Leigh, 2001	*Saguinus fuscicollis, Saguinus labiatus, Callimico goeldii*	identify both take-off and landing platform sizes	size and orientations of both take-off and landing platforms

Table 2 continued

Lawler and Stamps, 2002	*Alouatta palliata*	terminal branches = "distal, collective mass of branches where no single branch supports the weight of the animal"	use of terminal branches with tail use and without
Youlatos, 2002	*Ateles paniscus*	identifies locomotor modes that use multiple substrates (i.e., clamber), but does not quantify substrates	not included in analysis
Cant et al., 2003	*Ateles belzebuth, Lagothrix lagothrica*	identify support number (single or multiple) during brachiation and arm swing	support number used during stride and step phase
Thorpe and Crompton, 2005	*Pongo abelii*	identify number of supports: 1, 2, 3, 4, many	activities done on >1 substrate as well as mixed substrates
Lawler et al., 2006	*Callicebus brunneus, Callicebus torquatus*	terminal branches = "a small collective mass of distal branches that supports an animal where no single branch bears the majority of weight of the animal; branches are usually less than 0.5 cm in diameter"	percentage of use of "terminal" during feeding and foraging
Bezanson, 2009	*Alouatta palliata, Cebus capucinus*	identify single and multiple support use	percentage of feeding and foraging on different support characteristics
Jackson, 2011	*Cebuella pygmaea*	only for leaping- identify orientations of start and termination branches	orientations of takeoff and landing substrates
Manduell et al., 2011	*Pongo pygmaeus wurmbii*	identify number of supports: 1, 2, 3, 4, >4	frequency that animals use each number of supports
Myatt and Thorpe, 2011	*Pongo abelii*	record number of supports	analyzed effect of multiple support use on positional behavior
Workman and Schmitt, 2012	*Trachypithecus delacouri*	record locomotor transitions from different substrate types	analyze frequencies of transitions

Table 2 continued

Iurck et al., 2013	*Brachyteles hypoxanthus*	identify single and multiple support use	compare number of supports used to part of tree (terminal, central, or trunk), and activity (feed or rest)
Blanchard et al., 2015	*Lepilemur edwardsii*	used data from Warren and Crompton, 1997	(see above)
Furnell et al., 2015	*Propithecus verreauxi verreauxi*	record orientation, hight, and diameter of initial and terminal support of a bout	frequencies of locomotor modes that use multiple substrates

Table 3: Definitions of challenge types and number of occurrences in sample

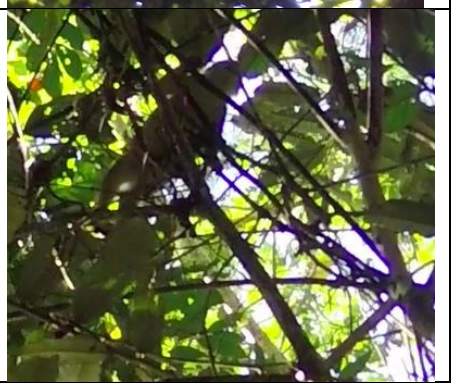
Challenge	**Description**	**N strides**	**Photo**
Gap	Animal crosses between two or more substrates in canopy that require the animal to reach, but maintains contact with the original substrate- making it not a leap. Photo: animal steps down from one substrate to another, closing the space between them by reaching.	23	
Ladder	Animal moves across multiple substrates lateral to the sagittal plane of its body. Photo: animal crosses several lateral supports that are close together.	7	

Table 3 continued

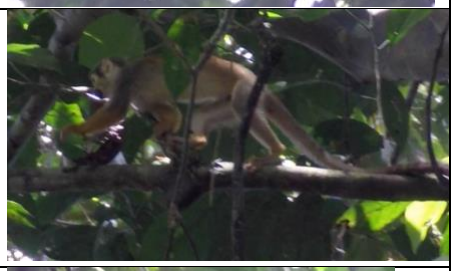
Parallel	Animal walks on two substrates parallel to each other, such as two lianas side-by-side, limbs stay on proper side of the body. Photo: left limbs and right limbs are on separate lianas that are next to each other for several touchdowns.	4	
Obstacle	Animal encounters interruption from main linear substrate, interruption crosses the main substrate and must either be stepped on or stepped over. Photo: animal steps on branch with right fore and hind that interrupts main linear support	5	
Offshoot	Branch, twig, liana, etc. protruding from main linear substrate that animal uses rather than keeping on main substrate. Photo: forelimbs use twig for support, while hindlimbs continue on main linear support.	17	
Linear transition	Animal is walking on main linear substrate, but transitions to another substrate in a linear progression, such as taking a fork in the branch. Photo: animal turns along linear supports to move along a single substrate, but substrate characteristics change as branches merge together at forks.	25	

Table 3 continued

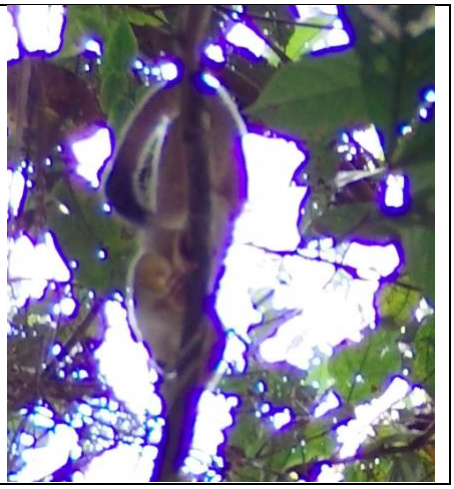
None	No challenge present for the limb, animal moves along a single substrate.	39	

Table 4: Definitions of gaits using APS model.

(from Abourachid, 2003)

Gait	**Fore Lag**	**Hind Lag**	**P Lag**
Pace	50	50	100
Trot	50	50	50
Lateral walk	50	50	>50
Diagonal walk	50	50	<50
Transverse gallop	<50	<50	Depends on the speed
Rotary gallop	<50	<0	Depends on the speed
Half bound	<50	0	Depends on the speed
Bound	0	0	Depends on the speed
Pronk	0	0	0

Table 5: Categorical gait results.

NA are strides that are missing a variable (hind lag, fore lag, or pair lag), and therefore categorical gait type could not be calculated. NAs were not removed from the dataset as other variables from the strides could be used for analysis.

Symmetry	**Gait**	**N**	**Percent**
Symmetrical	Lateral sequence	9	7.5%
Symmetrical	Diagonal sequence	36	30.0%
Asymmetrical	Ramble	65	54.17%
Asymmetrical	Other (DF<50)	5	4.17%
NA	NA	5	4.17%

Table 6: Effect of the presence of a challenge (type of challenge not included) on kinematic variables

Variable	**P value**	**Direction of effect**	**Significance**
Pair lag	0.03	>	**
Forelimb lag	0.02	>	**
Hindlimb lag	0.26	>	
Forelimb swing duration	0.32	>	
Hindlimb swing duration	0.49	>	
Forelimb stance duration	0.24	>	
Hindlimb stance duration	0.59	>	
Forelimb stride duration	0.47	>	
Hindlimb stride duration	0.06	>	approaching
Forelimb duty factor	0.25	>	
Hindlimb duty factor	0.55	>	

APPENDIX 2: FIGURES

Figure 1: Screenshot of BoutKeeper, Graphical User Interface in MATLAB.

Notice the Gait Diagram on the right indicating timing and temporal length of step events, as well as gray bars indicating challenges.

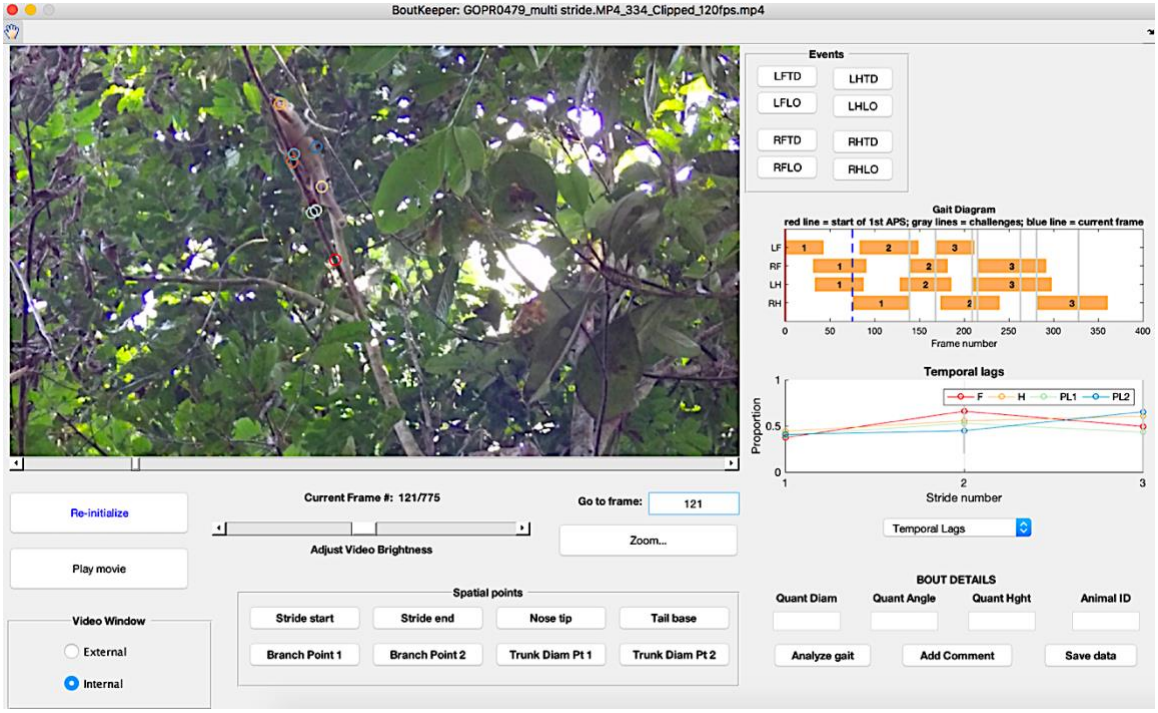
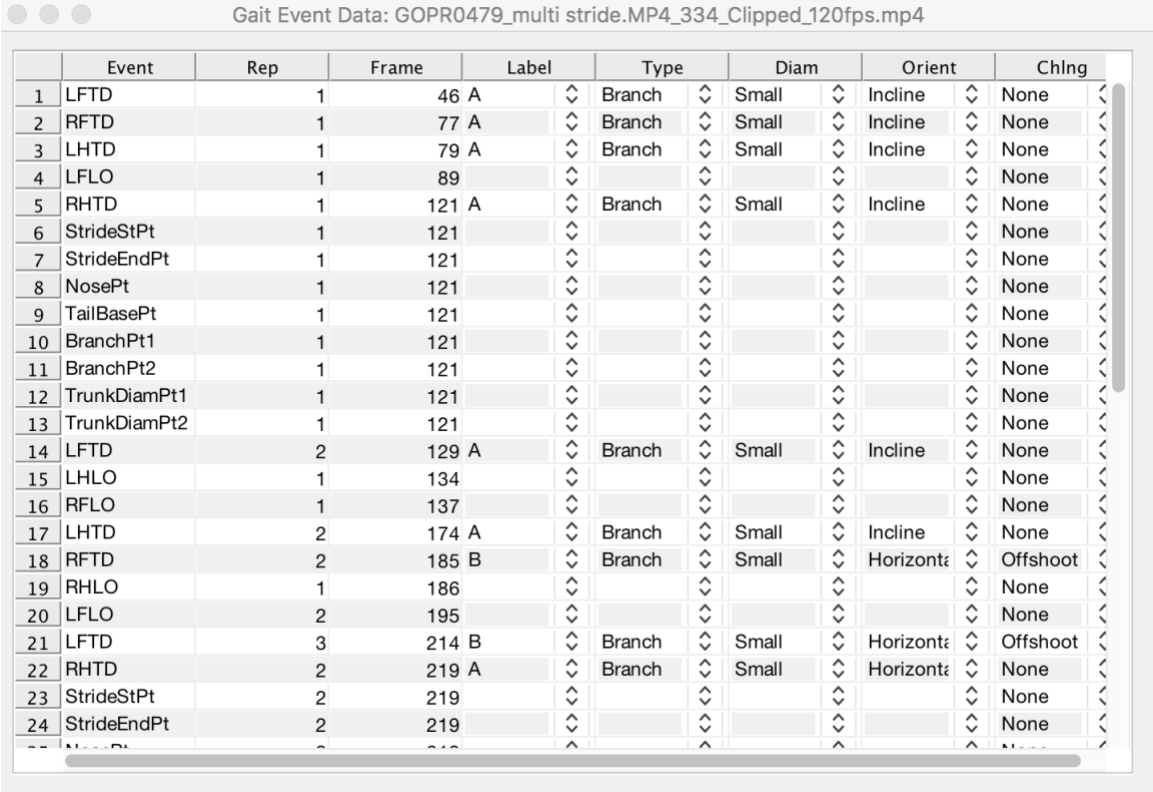


Figure 2: Screenshot of BoutKeeper program's data output.

Notice that columns have dropdown arrows, which can be interacted with to change substrate Label, Type, Diameter, Orientation, or Challenge Type. Also, the frame of each step event is given.



	Event	Rep	Frame	Label	Type	Diam	Orient	Chlng
1	LFTD	1	46	A	Branch	Small	Incline	None
2	RFTD	1	77	A	Branch	Small	Incline	None
3	LHTD	1	79	A	Branch	Small	Incline	None
4	LFLO	1	89					None
5	RHTD	1	121	A	Branch	Small	Incline	None
6	StrideStPt	1	121					None
7	StrideEndPt	1	121					None
8	NosePt	1	121					None
9	TailBasePt	1	121					None
10	BranchPt1	1	121					None
11	BranchPt2	1	121					None
12	TrunkDiamPt1	1	121					None
13	TrunkDiamPt2	1	121					None
14	LFTD	2	129	A	Branch	Small	Incline	None
15	LHLO	1	134					None
16	RFLO	1	137					None
17	LHTD	2	174	A	Branch	Small	Incline	None
18	RFTD	2	185	B	Branch	Small	Horizonta	Offshoot
19	RHLO	1	186					None
20	LFLO	2	195					None
21	LFTD	3	214	B	Branch	Small	Horizonta	Offshoot
22	RHTD	2	219	A	Branch	Small	Horizonta	None
23	StrideStPt	2	219					None
24	StrideEndPt	2	219					None

Figure 3: Frequency of temporal lead changes depending on the presence of a challenge.

Bar graph of association of temporal lead change presence depending on the presence or absence of a challenge in the stride. A challenge significantly affected the frequency of a temporal lead change occurring (p=0.05).

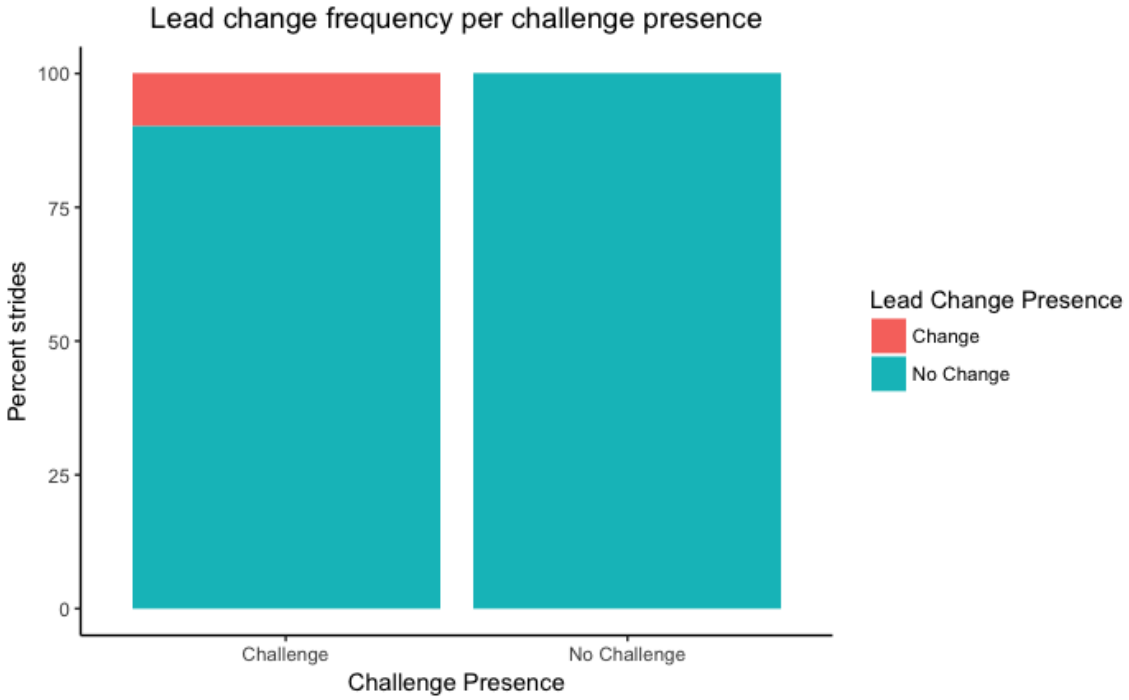


Figure 4: Frequency of temporal lead changes per challenge type.

Bar graph of association of temporal lead change presence across challenge types. Challenge type significantly impacted temporal lead change presence (p=0.05). However, no significant pairwise relationship existed between challenge types and temporal lead change.

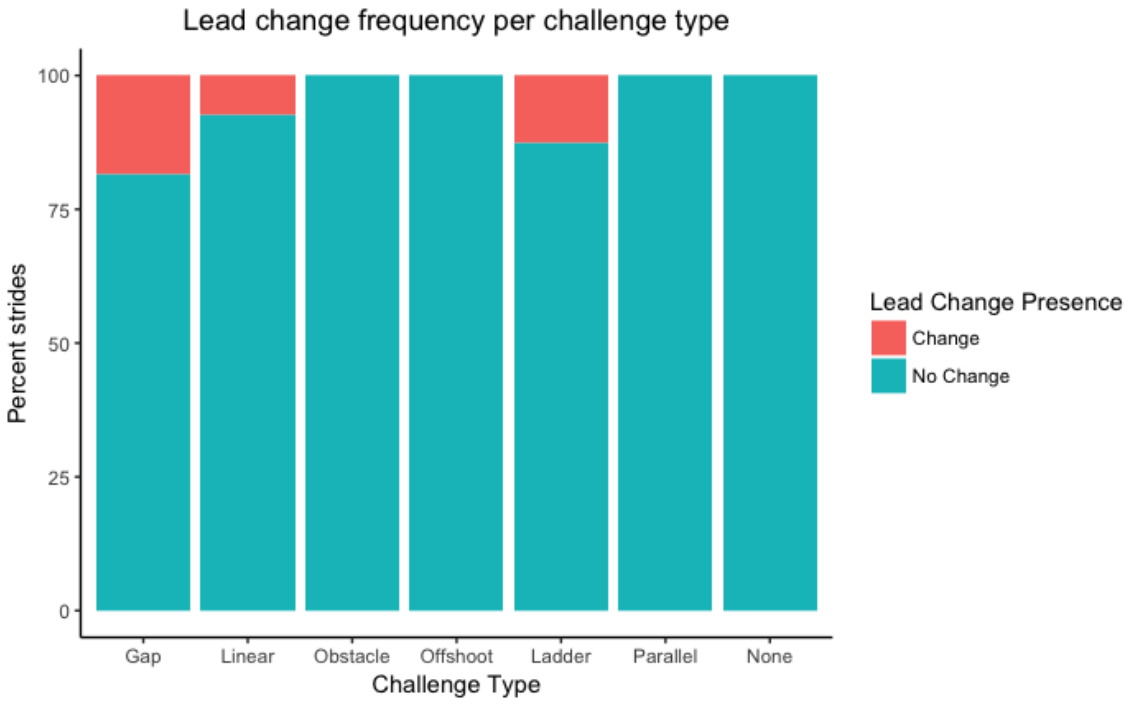


Figure 5: Frequency of temporal lead changes per grouped challenge types.

Bar graph of association of temporal lead change presence across grouped challenge types (Gap, Other, and None). Grouped challenge types significantly affected the frequency of temporal lead changes ($p<0.01$). Strides with gaps were significantly more likely to result in a lead change than strides with no challenges ($p=0.027$), and more likely to cause lead changes than other grouped challenge types, but not significantly so ($p=0.08$).

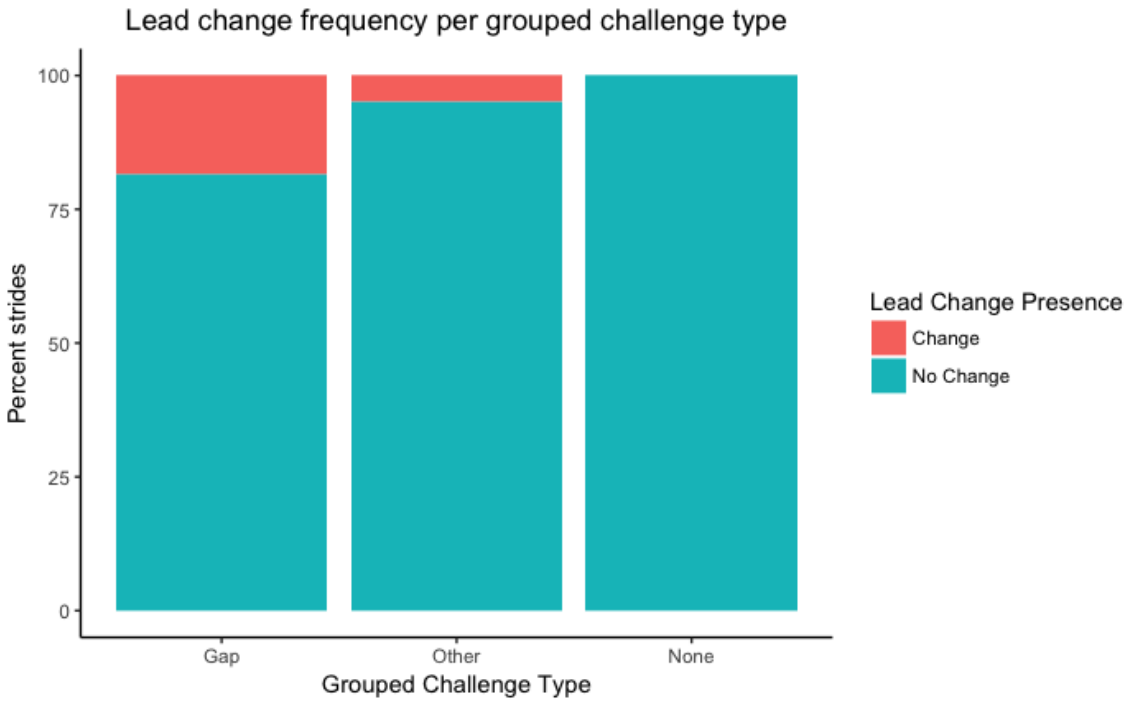


Figure 6: Effect of the presence of a challenge on pair lag.

The box and whiskers plot of the distribution of pair lag across strides with challenges and no challenges in which the horizontal line represents the median, the box represents interquartile range, the whiskers represent 150% of the interquartile range (IQR), the points represent outliers (>150% IQR).
Pair lag significantly increases in strides with a challenge as opposed to strides without a challenge within the same bout (p=0.03).

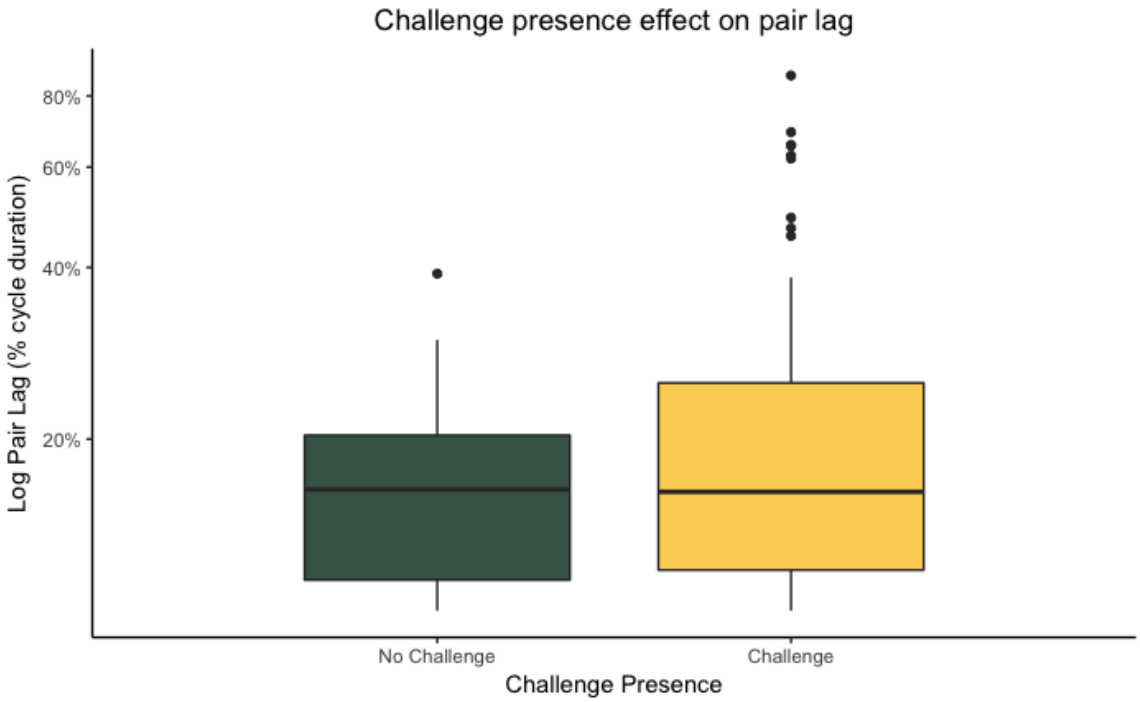


Figure 7: Effect of the presence of challenge on fore lag.

The box and whiskers plot of the distribution of fore lag across strides with challenges and no challenges in which the horizontal line represents the median, the box represents interquartile range, the whiskers represent 150% of the interquartile range (IQR), the points represent outliers (>150% IQR).
Fore lag significantly increases in strides with a challenge as opposed to strides without a challenge within the same bout (p=0.02).

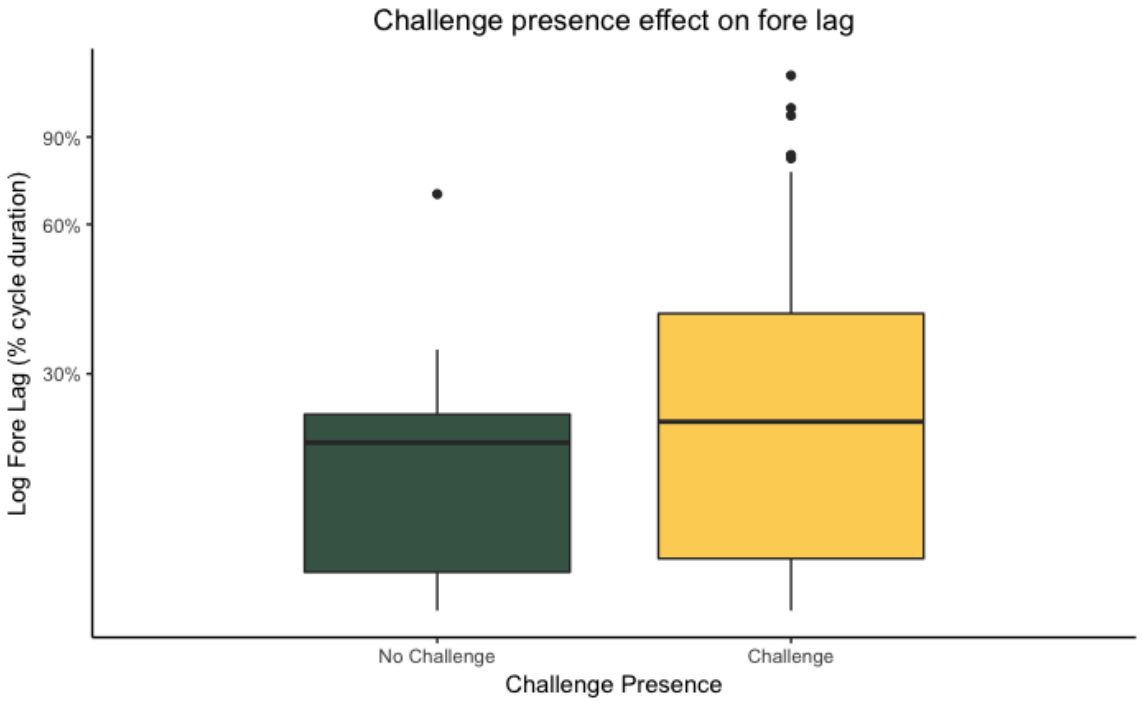


Figure 8: Type of challenge effect on pair lag.

The box and whiskers plot of the distribution of pair lag change across challenge type in which the horizontal line represents the median, the box represents interquartile range, the whiskers represent 150% of the interquartile range (IQR), the points represent outliers (>150% IQR).
Type of challenge had no significant impact on pair lag chance (p>0.05)

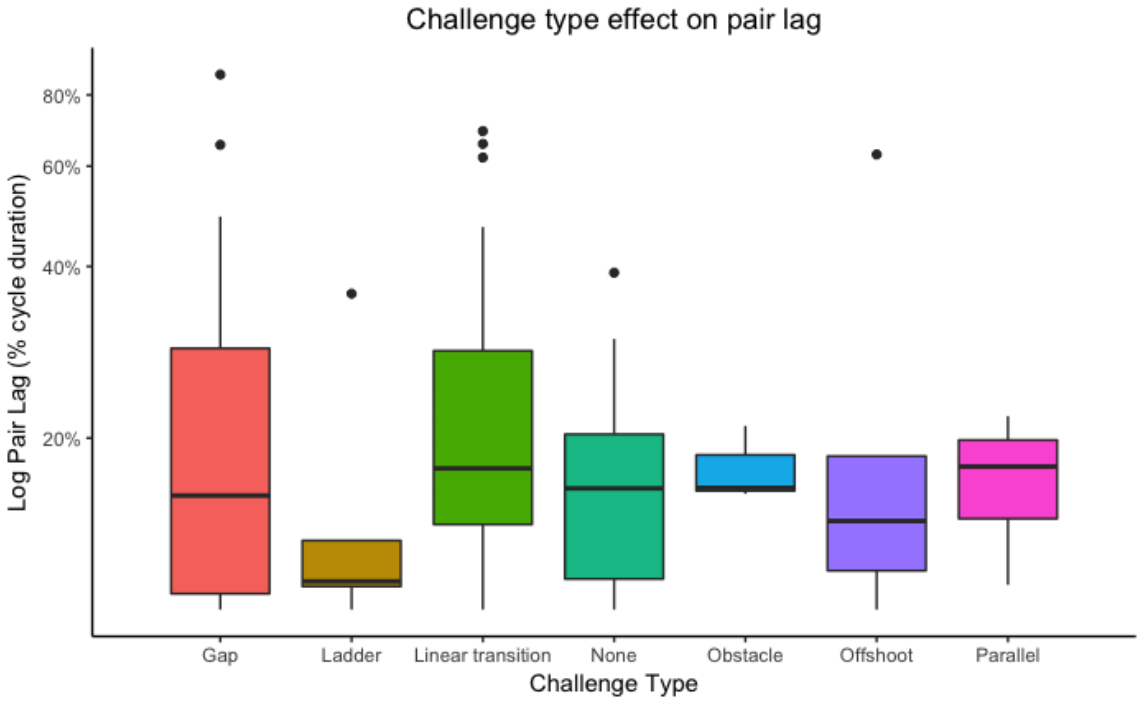


Figure 9: Type of challenge effect on fore lag.

The box and whiskers plot of the distribution of fore lag change across challenge type in which the horizontal line represents the median, the box represents interquartile range, the whiskers represent 150% of the interquartile range (IQR), the points represent outliers (>150% IQR).
Type of challenge had no significant impact on fore lag change (p>0.05)

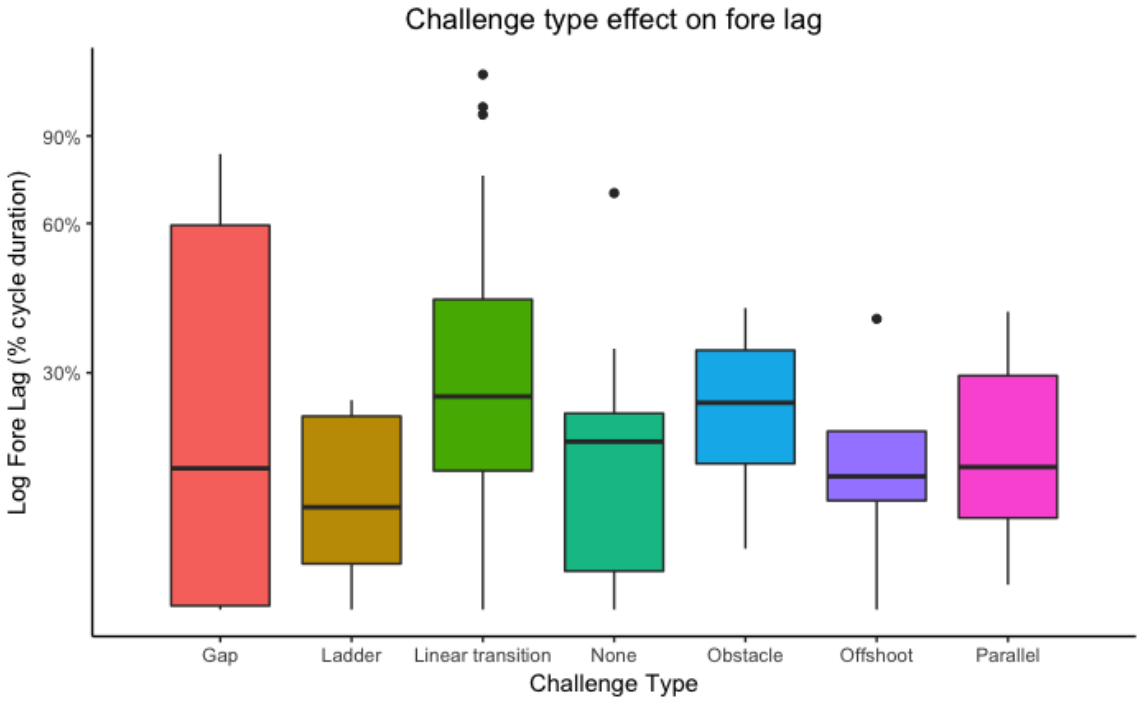


Figure 10: Grouped challenges effect on pair lag.

The box and whiskers plot of the distribution of pair lag change across grouped challenge type in which the horizontal line represents the median, the box represents interquartile range, the whiskers represent 150% of the interquartile range (IQR), the points represent outliers (>150% IQR).
When challenges were grouped into Gap, Other, and None, the effect on pair lag approached significance (p=0.075).

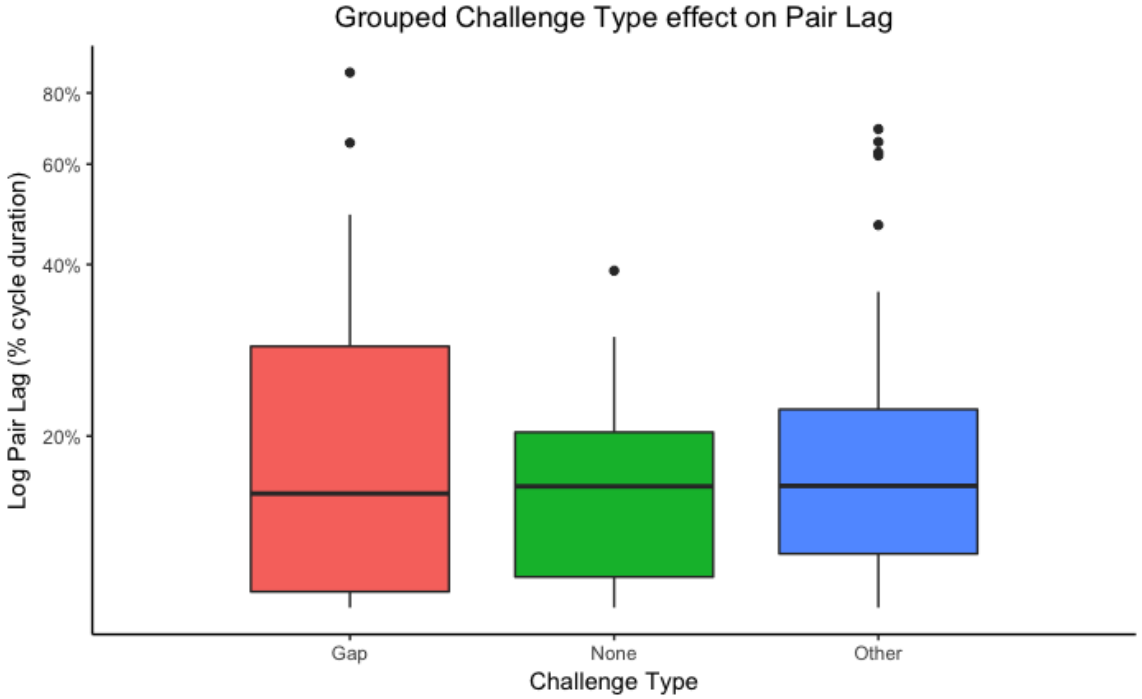
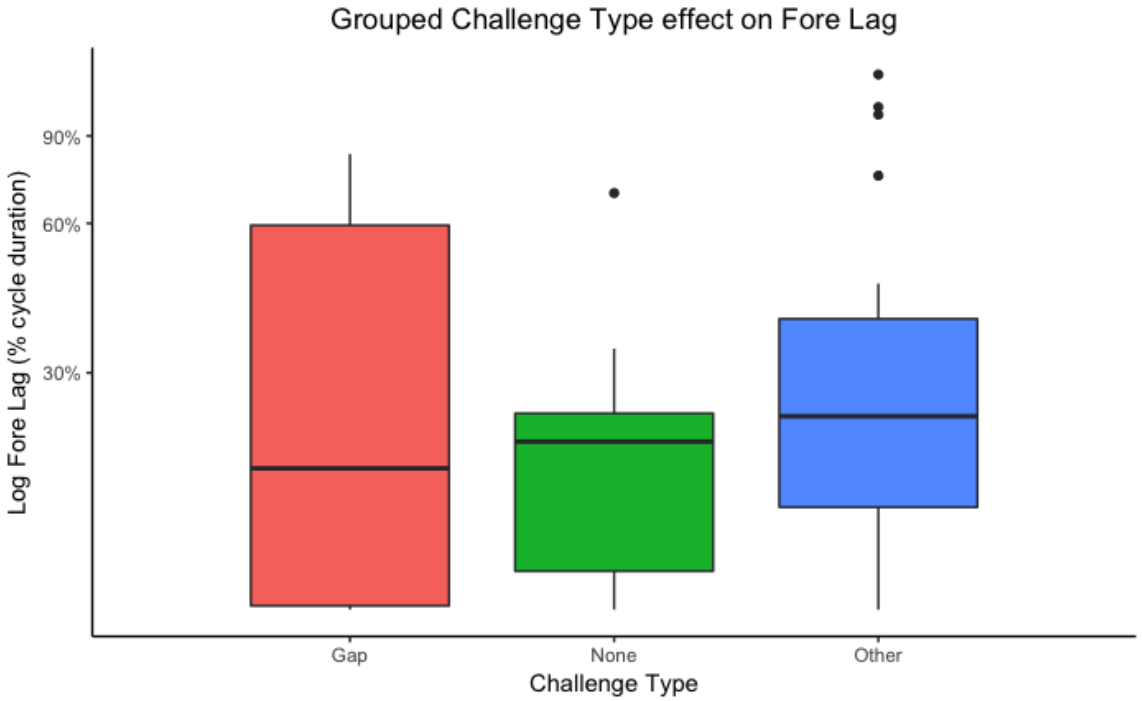


Figure 11: Grouped challenges effect on fore lag.

The box and whiskers plot of the distribution of fore lag change across grouped challenge type in which the horizontal line represents the median, the box represents interquartile range, the whiskers represent 150% of the interquartile range (IQR), the points represent outliers (>150% IQR).
When challenges were grouped into Gap, Other, and None, the effect on fore lag approached significance (p=0.06).



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